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EFFECT OF ENVIRONMENTAL FACTORS  
ON OXIDIZING ENZYMES OF  
ROSE MALLOW SEEDS

A DISSERTATION SUBMITTED TO THE FACULTY  
OF THE DIVISION OF THE BIOLOGICAL SCIENCES  
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF  
PHILOSOPHY

DEPARTMENT OF BOTANY  
1937

By  
KATHRYN E. STALEY

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## EFFECTS OF ENVIRONMENTAL FACTORS ON OXIDIZING ENZYMES OF ROSE MALLOW SEEDS

KATHRYN STALEY

(WITH EIGHT FIGURES)

### Introduction

The power of plant tissues to break up hydrogen peroxide into water and molecular oxygen was known long before catalase was recognized as an enzyme, discovered, and named by LOEW (18) in 1901. This discovery opened a new field for investigation and many studies were undertaken. The wide distribution of catalase in plant and animal tissue led LOEW to conclude that it might serve as a protective measure against the injurious protoplasmic effects of hydrogen peroxide and possibly other related compounds formed in respiration. DIXON (8) has shown that when purine bases are oxidized by molecular oxygen in the presence of xanthine-oxidase as a catalyst, the oxidase undergoes destruction during the course of the reaction owing to the hydrogen peroxide that is formed. He found that this destruction could be prevented by the addition of catalase. Pneumococci in contact with a free supply of oxygen produced hydrogen peroxide in sufficient amount to inhibit their growth, but if catalase were added the bacteria grew vigorously.

A close correlation between catalase activity and respiration was found in potatoes (2), in rice grains germinated under water (22), and in sweet corn (3). CROCKER and HARRINGTON (6) found this same correlation in the seeds of Johnson grass but not in Amaranthus. RHINE (26) using a number of different seeds concluded that catalase activity is a measure of the metabolic activity only when there is a rapid change in respiration. The absence of catalase activity in certain blue green algae (14) and in anaerobic bacteria (20) indicates that catalase is not essential to the respiration of all cells. McLEOD and GORDON (20) consider it possible that catalase destroys some injurious substance such as  $H_2O_2$  which is produced by aerobic organisms and by anaerobic organisms in the presence of oxygen; thus anaerobic organisms lacking catalase would be injured when exposed to oxygen.

A variation in catalase activity was observed by POPE (24, 25) in the different parts of the same barley plant. The relation of the catalase activity to physiological breakdown in Jonathan apples convinced NELLER (23) that catalase activity could be used as a true index of the rate of metabolic activity. HEINICKE (15, 16) concluded that catalase activity is a highly sensitive index to changes in the internal condition of the apple tree, and that this activity varies with the season, with the part of the tree tested, and with

various environmental factors. He believed that the catalase activity would eventually prove as good an indicator of metabolism as do the temperature and pulse rate in the human body.

The detailed nature of catalase has been carefully investigated. LOEW (18) recognized an insoluble and a soluble form. He thought the insoluble form, or alpha catalase, was a compound of the soluble form with a nucleoprotein. The soluble form, beta catalase, was considered an albumose which could be liberated by the action of a very dilute alkaline medium upon the insoluble form. APPLEMAN (1) was able to separate the two forms by the use of ordinary filter paper, but could not do so with a Chamberland-Pasteur filter. Approximately fifty per cent. of the total enzyme content passed through the filter paper, indicating that the two forms were present in about equal amounts.

For the chemical analysis of catalase, liver has served as the most convenient source of the catalase preparation. EULER and JOSEPHSON (11) state that no relationship exists between the activity of the preparation and the percentage of iron present. They analyzed two preparations which had a catalytic activity of 43,000 and 40,000 respectively, and found that the former contained 0.63 per cent. iron while the latter contained 0.15 per cent. ZEILE and HELLSTRÖM (32) found that a strong preparation of catalase obtained from horse liver had a distinct absorption spectrum corresponding to a hematin compound. Regardless of degree of purity they thought there was a strict proportionality between the catalytic activity and the concentration of the porphyrin-bound iron. Comparing the value calculated for hemin with that for catalase they found that in the catalase union the iron was decidedly more active than the usual porphyrin-bound iron. They concluded that the porphyrin-iron complex in catalase must be bound in a very specific way to the protein or some other colloidal carrier, which binding confers to it this unusual increase of activity. They also found that such factors as high temperature, alkali, acids, or the addition of small amounts of KCN or  $\text{H}_2\text{S}$ , which abolished or inhibited the catalase activity, also modified the absorption spectrum of the hematin. STERN (27) studying the activity of ferrous and ferric ions, hemin, and catalase, respectively, in decomposing  $\text{H}_2\text{O}_2$ , found that the binding of iron into porphyrin resulted in a greater catalytic activity than for iron alone; and a still greater activity was obtained when hemin was coupled with catalase protein. The absorption spectrum and pH observations led STERN (27, 28) to conclude that the parahematin compounds were closely related to natural catalase. It has been estimated that the diameter of a catalase particle is 5.46  $\mu$  and that the molecular weight of catalase is approximately 68,900. This indicates that the molecules are similar in size to those of hemoglobin. Horse liver catalase has been split by dialysis into two inactive components, one of low molecular



weight (possibly hemin) and the other possibly protein, which, upon being mixed become active. TAUBER and KLEINER (30) concluded that catalase solutions could be digested by trypsin.

Crystalline catalase prepared from beef liver (29) coagulated upon heating and showed many other properties characteristic of proteins. The investigators report that the pyridine hemochromogen test was readily obtained with this preparation. It appears, then, that catalase is similar to peroxidase (13) and oxidase (13, 31, 44) in having an iron-porphyrin complex as the active group.

The catalase studies reported here were undertaken to determine the effect of environmental factors such as temperature, exposure to gases, and the submergence of seeds in water upon the activity of this iron porphyrin complex of imbibed seeds. Other oxidizing enzymes also were studied to obtain a more generalized idea of the effect of such conditions on the enzyme complexes associated with oxidation in the plant.

In biological oxidations, peroxidases are thought to transfer the oxygen, linked as a peroxide, to oxidizable substances. They cannot decompose  $H_2O_2$  in the absence of an oxidizable substance. The oxidases render such matter more susceptible to reduction.

### Materials and methods

Seeds of the Malvaceae are noted for their hardness and longevity. Two varieties of rose mallow, or *Hibiscus*, were selected: the variety Golden Bowl, and variety Dark Red. The seeds of the former are larger than those of the latter and are approximately twice as heavy; both are very erratic in germination because of their impervious seed coats. Seeds of the 1936 crop of Dark Red and of the 1934 and 1936 crop of Golden Bowl were obtained from Henry Dreer Seed Co., Philadelphia, Pa. Only a small quantity of the 1936 crop of Golden Bowl was available.

In order to secure greater uniformity in germination the seeds were subjected to the action of  $H_2SO_4$  (sp. gr. 1.84) for 53 minutes, washed with sterile water, and the acid neutralized with lime water. They were placed in sterile water until they showed evidence of swelling, disinfected with 0.25 per cent. solution of uspulín for one hour, and thoroughly washed with sterile water. The uspulín treatment was inadequate for the seeds which were kept under water for 30 days, consequently, a more effective method of disinfection was used. This treatment consisted of soaking the seeds 4 hours in a 1:30 dilution of Zonite and rinsing in sterile water before placing them under the conditions of the experiment.

All glassware, cellucotton, filter paper, and water used for the experiments were sterilized in the autoclave at 15 lb. pressure for 30 min. on each of two successive days. A modified APPLEMAN (1) apparatus was used for

catalase determinations; the liberation of oxygen from neutralized Merck's hydrogen peroxide served as a measure of the catalase activity. Each bottle was standardized by permanganate titration before using, and the equivalent of 10 ml. was used for each determination. The tissue was ground for 5 min. in a mortar with clean sand, a small excess of  $\text{CaCO}_3$ , and a measured amount of distilled water. The material was then washed into the shaking bottle with the remainder of the water previously measured. The equivalent of 10 ml. of water was used for each determination. The bottle was fastened in the shaker in such a manner that the former was suspended in a water bath maintained at a temperature of  $20^\circ \text{C}$ . When the contents of the bottle were of the same temperature as the water bath, the two-holed stopper fitted with dropping funnel and delivery tube was adjusted to position, and 10 ml. of neutralized  $\text{H}_2\text{O}_2$  was added 15 seconds before the shaking began. The levelling bulb was used to maintain pressure equilibrium. Readings were taken at 1, 2, 3, 5, and 10 minutes. The first four readings served as a check on the final reading and showed the rate at which the molecular oxygen was evolved. The results were corrected for standard conditions of temperature and pressure.

Simplified Bunsen tubes and a mechanical shaker were used for oxidase determinations. The tissues were ground for 5 min. in a mortar with a very small quantity of distilled water. The ground tissue was then washed into a small graduated calibrated cylinder. The volume was brought to 4 ml. and the contents thoroughly mixed. One ml. of this mixture was pipetted into the short arm, and 5 ml. of a 1 per cent. solution of pyrogallol into the larger arm, of the Bunsen tube; care was taken not to mix the two until the shaker was started. The top was adjusted to leave the contents open to the outside air, while the tube was fastened into the mechanical shaker and allowed to stand until temperature equilibrium had been established: The top was then turned to prevent contact with the outside air and the shaker, adjusted to make one hundred complete excursions per minute, set in motion. Readings were made at 15-minute intervals for the first hour and at thirty-minute intervals for the second hour. At the end of the first hour the manometers were opened and reset. The Bunsen tubes were calibrated, and the results corrected for 25 ml. volume and standard conditions of temperature and pressure.

## Results

### TEMPERATURE EFFECTS ON CATALASE

Imbibed seeds of the two varieties tested were placed on moist cellulose and filter paper in sterile Petri dishes. The seeds were subjected to temperatures of  $8^\circ$ ,  $20^\circ$ ,  $37^\circ$ ,  $42^\circ$ , and  $47^\circ \text{C}$ . for a period of seven days. Catalase determinations in duplicate were made daily. Six seeds of variety Golden Bowl



and twelve seeds of Dark Red, respectively, were used in a determination. All results were corrected to standard conditions.

Seeds of the 1934 crop of Golden Bowl subjected to temperatures of 8°, 20°, and 27° C. show a decrease in catalase activity, followed by an increase until the food is exhausted, at which time the catalase decreases (table IA).

TABLE I

TEMPERATURE EFFECTS ON CATALASE ACTIVITY OF HIBISCUS SEEDS.  
MILLILITERS OF OXYGEN LIBERATED IN TEN MINUTES

|                                                | DAYS | TEMPERATURE |            |            |            |            |            |
|------------------------------------------------|------|-------------|------------|------------|------------|------------|------------|
|                                                |      | 8° C.       | 20° C.     | 27° C.     | 37° C.     | 42° C.     | 47° C.     |
|                                                |      | <i>ml.</i>  | <i>ml.</i> | <i>ml.</i> | <i>ml.</i> | <i>ml.</i> | <i>ml.</i> |
| A<br><br>Golden Bowl<br>Six seeds of 1934 crop | 0    | 39.6        | 39.6       | 39.6       | 39.6       | 39.6       | 39.6       |
|                                                | 1    | 41.2        | 32.1       | 34.4       | 40.2       | 30.2       | 0.9        |
|                                                | 2    | 40.3        | 40.5       | 34.8       | 40.2       | 9.7        | 0.0        |
|                                                | 3    | 29.7        | 71.0       | 66.9       | 36.3       | 14.8       | 0.0        |
|                                                | 4    | 33.6        | 52.8       | 36.0       | 31.5       | 4.5        | 0.0        |
|                                                | 5    | 42.6        | 76.6       | 55.4       | 14.2       | 4.3        | 0.0        |
|                                                | 6    | 44.6        | 82.8       | 79.0       | 6.0        | 1.9        | 0.0        |
|                                                | 7    | 45.9        | 55.6       | 97.1       | 4.8        | 1.0        | 0.0        |
| B<br><br>Golden Bowl<br>Six seeds of 1936 crop | 0    | 36.6        | 36.6       | 36.6       | 36.6       | 36.6       | 36.6       |
|                                                | 1    | 40.3        | 40.9       | 35.9       | 29.3       | 15.6       | 0.5        |
|                                                | 2    | 32.6        | 33.9       | 34.6       | 28.2       | 16.8       | 0.4        |
|                                                | 3    | 33.0        | 38.4       | 45.4       | 15.5       | 7.1        | 0.4        |
|                                                | 4    | 29.3        | 42.7       | 45.6       | 14.1       | 5.7        | 0.3        |
|                                                | 5    | 32.2        | 48.5       | 65.9       | 13.4       | 4.7        | 0.2        |
|                                                | 6    | 28.3        | 59.5       | 109.6      | 13.5       | 2.2        | 0.1        |
|                                                | 7    | 30.9        | 69.8       | 93.5       | 5.8        | 1.4        | 0.1        |
| C<br><br>Dark Red<br>Twelve seeds of 1936 crop | 0    | 38.8        | 38.8       | 38.8       | 38.8       | 38.8       | 38.8       |
|                                                | 1    | 26.6        | 19.4       | 20.6       | 22.4       | 27.0       | 0.0        |
|                                                | 2    | 28.0        | 46.2       | 57.4       | 35.5       | 19.5       | 0.0        |
|                                                | 3    | 39.7        | 46.8       | 35.1       | 31.8       | 4.8        | 0.0        |
|                                                | 4    | 23.9        | 71.7       | 97.5       | 38.4       | 12.9       | 0.0        |
|                                                | 5    | 22.6        | 91.5       | 105.3      | 38.5       | 8.6        | 0.0        |
|                                                | 6    | 19.2        | 97.6       | 104.7      | 33.1       | 6.4        | 0.0        |
|                                                | 7    | 24.5        | 109.4      | 86.5       | 29.5       | 4.9        | 0.0        |

The drop in catalase content is slower in the seeds kept at 8° C. since it takes place on the fourth day; at 20° and 27° C. the depression occurs on the first day. At 37° C. there is noted a very slight increase on the first and second day (the depression may have come earlier than the 24-hour limit) followed by a gradual decrease in catalase content. The seeds at this temperature gave no visible signs of germination. At 42° C. there is a slight decrease on the first day followed by a rapid drop in catalase activity. At 47° C. the decrease the first day is very rapid and soon reaches zero.

The catalase activity of the 1936 crop of variety Golden Bowl agrees rather closely with that of the 1934 crop (table IB). At 47° C. the catalase activity shows a slightly greater resistance to this temperature than that

of the 1934 crop. The results obtained indicate that the difference in age in these particular seeds is of no significance in catalase activities.

The seeds of variety Dark Red and Golden Bowl respond in a similar fashion in both germination and catalase activity (table IC, fig. 1, 2).

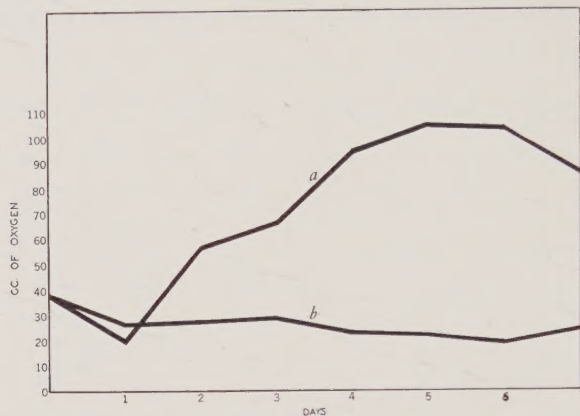


FIG. 1. The effect of temperature on the catalase activity of imbibed seeds of Hibiscus variety Dark Red. (a) 27° C., and (b) 8° C.

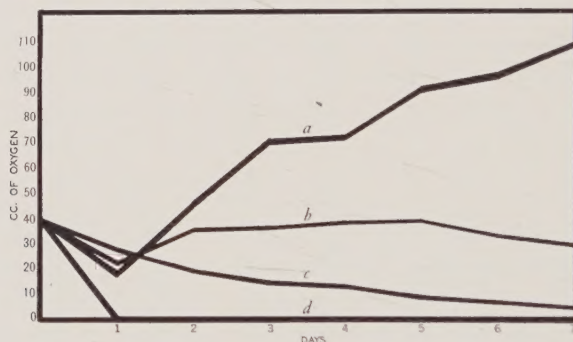


FIG. 2. The effect of temperature on the catalase activity of imbibed seeds of Hibiscus variety Dark Red. (a) 20° C., (b) 37° C., (c) 42° C., and (d) 47° C.

At 8°, 20°, 27°, and 37° C. there is a characteristic decline in catalase activity in the early stages of germination, followed by an increase. This decrease occurs later with seeds kept at 8° C. than with those at the higher temperatures. Good germination occurred at 20° and 27° C., while only a few were able to germinate at 37° C. Apparently this accounts for the greater activity of the catalase at 37° C. as compared with that of the Golden Bowl seeds at the same temperature. At 42° C. the seeds gave no indication of germination and there was a gradual decrease in the catalase activity. At 47° C. the catalase was completely inactivated during the first day.



The results indicate that the catalase activity follows a definite course. In the early stages of germination the activity is depressed, but increases as germination progresses, until the food supply is apparently exhausted.

#### THE EFFECT OF OXYGEN AND NITROGEN ON CATALASE

In order to determine the effect of oxygen and nitrogen on the catalase activity of imbibed seeds, swollen seeds of both species were placed on moist cellulocotton and filter paper in a series of 8-oz. bottles. Each bottle contained a number of seeds sufficient for seven daily determinations. The bottles were connected and one series subjected to a steady stream of oxygen; the second series was subjected to a steady stream of nitrogen. The bottles were kept in the basement at a room temperature of 25°–27° C. Six seeds of Golden Bowl and twelve seeds of Dark Red, (except where indicated) were used in each determination.

TABLE II

CATALASE ACTIVITY OF HIBISCUS SEEDS TREATED WITH OXYGEN. MILLILITERS OF OXYGEN LIBERATED IN MINUTES ON SEVEN CONSECUTIVE DAYS

| DAYS | MINUTES                  |            |            |            |            |                          |            |            |            |            |
|------|--------------------------|------------|------------|------------|------------|--------------------------|------------|------------|------------|------------|
|      | SIX SEEDS OF GOLDEN BOWL |            |            |            |            | TWELVE SEEDS OF DARK RED |            |            |            |            |
|      | 1                        | 2          | 3          | 5          | 10         | 1                        | 2          | 3          | 5          | 10         |
|      | <i>ml.</i>               | <i>ml.</i> | <i>ml.</i> | <i>ml.</i> | <i>ml.</i> | <i>ml.</i>               | <i>ml.</i> | <i>ml.</i> | <i>ml.</i> | <i>ml.</i> |
| 0    | 6.7                      | 14.6       | 20.7       | 23.0       | 39.6       | 9.2                      | 14.8       | 19.4       | 26.8       | 38.8       |
| 1    | 5.7                      | 11.8       | 15.7       | 20.6       | 30.4       | 7.3                      | 11.8       | 17.1       | 23.8       | 34.0       |
| 2    | 10.9                     | 16.9       | 21.4       | 28.1       | 37.6       | 13.1                     | 22.0       | 33.3       | 45.0       | 69.1       |
| 3    | 12.1                     | 20.9       | 28.3       | 39.1       | 57.1       | 21.4                     | 35.4       | 46.9       | 64.1       | 89.7       |
| 4    | 11.7                     | 20.6       | 27.6       | 38.9       | 57.8       | 35.3                     | 60.4       | 79.0       | 108.4      | 152.9*     |
| 5    | 15.1                     | 31.7       | 36.0       | 52.6       | 75.1       | 34.9                     | 58.1       | 78.6       | 109.5      | 160.8*     |
| 6    | 17.6                     | 28.3       | 36.9       | 51.9       | 76.5       | 30.1                     | 49.7       | 64.4       | 88.7       | 127.4*     |
| 7    | 6.1                      | 9.9        | 14.1       | 20.9       | 33.7       | 30.0                     | 49.0       | 62.9       | 86.6       | 118.3*     |

\* Owing to the high catalase activity, 6 seeds were used for each determination and four determinations made so that the number given above is the amount of O<sub>2</sub> evolved by 12 seeds.

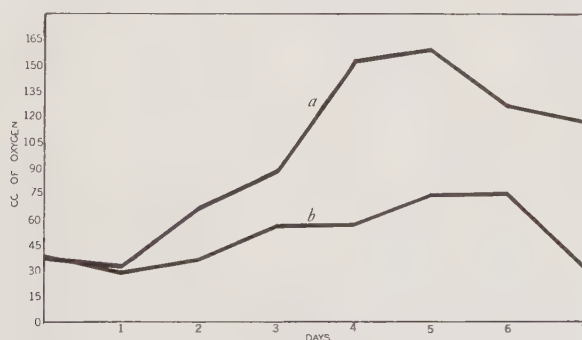


FIG. 3. The effect of oxygen on the catalase activity of imbibed seeds of (a) variety Dark Red and (b) variety Golden Bowl. Based on 10-minute data.

The results given in table II and figure 3 are for 1, 2, 3, 5, and 10 minutes. This shows the rate at which the oxygen is evolved as well as the final reading. The results given indicate that the seeds of the two species treated with oxygen show the same general course of catalase activity which is similar to that obtained in the seeds subjected to temperatures favorable for germination. The seeds of both species show a decrease in catalase on the first day, with an increase reaching the peak for variety Dark Red on the 5th day and for Golden Bowl on the 6th day, followed by a decrease. The conditions were favorable for good germination.

The results of the treatment with nitrogen are essentially the same as those obtained with oxygen (table III, fig. 4). The amount of increase in

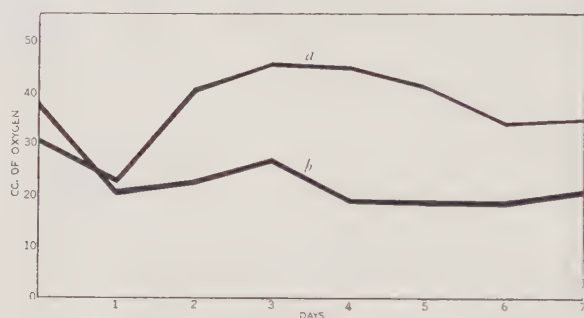


FIG. 4. The effect of nitrogen on the catalase activity of imbibed seeds (a) Golden Bowl and (b) Dark Red. Based on 10-minute data.

catalase activity is less, however, and it was not completely inactivated during the seven day period. Only the initial stages of germination were noted since the absence of oxygen precluded the possibility of the usual reaction.

TABLE III

CATALASE ACTIVITY OF HIBISCUS SEEDS TREATED WITH NITROGEN. MILLILITERS OF OXYGEN LIBERATED IN MINUTES ON SEVEN CONSECUTIVE DAYS

| DAYS | MINUTES                  |      |      |      |      |                          |      |      |      |      |
|------|--------------------------|------|------|------|------|--------------------------|------|------|------|------|
|      | SIX SEEDS OF GOLDEN BOWL |      |      |      |      | TWELVE SEEDS OF DARK RED |      |      |      |      |
|      | 1                        | 2    | 3    | 5    | 10   | 1                        | 2    | 3    | 5    | 10   |
|      | ml.                      | ml.  | ml.  | ml.  | ml.  | ml.                      | ml.  | ml.  | ml.  | ml.  |
| 0    | 6.7                      | 14.7 | 20.7 | 23.0 | 31.0 | 9.2                      | 14.8 | 19.4 | 26.8 | 38.8 |
| 1    | 4.9                      | 10.3 | 13.6 | 19.0 | 23.8 | 4.9                      | 8.4  | 11.6 | 15.6 | 21.3 |
| 2    | 11.0                     | 18.7 | 25.0 | 32.5 | 40.8 | 5.1                      | 8.7  | 12.2 | 16.7 | 23.1 |
| 3    | 12.1                     | 20.6 | 27.2 | 35.6 | 45.5 | 5.2                      | 9.5  | 13.1 | 18.8 | 27.3 |
| 4    | 6.8                      | 14.7 | 19.6 | 29.6 | 45.3 | 4.5                      | 7.7  | 10.3 | 14.1 | 19.4 |
| 5    | 7.6                      | 14.8 | 21.3 | 33.7 | 40.9 | 4.0                      | 6.6  | 9.6  | 13.6 | 19.2 |
| 6    | 7.2                      | 13.2 | 18.4 | 25.5 | 34.4 | 3.7                      | 6.7  | 9.3  | 13.6 | 19.4 |
| 7    | 6.9                      | 11.7 | 16.4 | 23.1 | 35.0 | 3.6                      | 6.7  | 9.4  | 15.3 | 20.8 |



## CATALASE ACTIVITY OF SUBMERGED SEEDS

Imbibed seeds were placed in a series of plugged tubes containing sterile distilled water to a depth of 5, 100, and 155 mm. The seeds were kept at room temperature for a period of thirty days and catalase determinations were made daily. Six seeds of Golden Bowl and 12 seeds of Dark Red were used in the determinations. Under all conditions there was a decrease in catalase activity in the early part of the time period then a slight increase followed by a marked decrease (table IV). Figure 5 shows the course of the catalase activity of the 1934 crop of Golden Bowl at the various depths. The embryos were able to undergo the early stages of germination and a few pushed the radicle through the seed coat. The rate and amount of decrease

TABLE IV

CATALASE ACTIVITY OF HIBISCUS SEEDS SUBMERGED IN WATER. MILLILITERS OF OXYGEN LIBERATED IN TEN MINUTES ON THIRTY CONSECUTIVE DAYS

| DAYS | GOLDEN BOWL, 6 SEEDS |            |            |             |            |            | DARK RED, 12 SEEDS |            |            |
|------|----------------------|------------|------------|-------------|------------|------------|--------------------|------------|------------|
|      | 1934 CROP            |            |            | 1936 CROP   |            |            | 1936 CROP          |            |            |
|      | WATER DEPTH          |            |            | WATER DEPTH |            |            | WATER DEPTH        |            |            |
|      | 5 MM.                | 100 MM.    | 155 MM.    | 5 MM.       | 100 MM.    | 155 MM.    | 5 MM.              | 100 MM.    | 155 MM.    |
|      | <i>ml.</i>           | <i>ml.</i> | <i>ml.</i> | <i>ml.</i>  | <i>ml.</i> | <i>ml.</i> | <i>ml.</i>         | <i>ml.</i> | <i>ml.</i> |
| 0    | 45.0                 | 45.0       | 45.0       | 47.0        | 47.0       | 47.0       | 39.0               | 39.0       | 39.0       |
| 1    | 35.6                 | 44.4       | 28.3       | 30.8        | 40.4       | 37.7       | 22.6               | 13.8       | 21.5       |
| 2    | 45.7                 | 37.3       | 39.7       | 36.1        | 39.6       | 41.7       | 25.0               | 23.0       | 23.7       |
| 3    | 44.5                 | 46.6       | 50.0       | 43.4        | 40.4       | 29.6       | 19.7               | 20.7       | 22.2       |
| 4    | 42.8                 | 50.5       | 41.7       | 41.7        | 40.5       | 42.4       | 30.4               | 20.9       | 21.9       |
| 5    | 42.1                 | 43.2       | 56.5       | 34.5        | 39.2       | 37.6       | 26.9               | 33.6       | 24.9       |
| 6    | 53.3                 | 41.9       | 42.1       | 27.6        | 43.7       | 37.5       | 21.5               | 25.4       | 19.3       |
| 7    | 43.6                 | 37.4       | 43.1       | 43.6        | 41.7       | 39.7       | 22.8               | 16.3       | 18.2       |
| 8    | 33.6                 | 33.9       | 36.3       | 43.6        | 34.5       | 32.9       | 23.2               | 27.5       | 21.3       |
| 9    | 27.5                 | 19.6       | 44.0       | 48.5        | 33.2       | 38.6       | 22.7               | 20.2       | 21.1       |
| 10   | 31.5                 | 32.8       | 43.9       | 44.7        | 36.3       | 40.1       | 20.9               | 20.2       | 17.4       |
| 11   | 34.4                 | 19.9       | 22.9       | 44.1        | 34.5       | 41.3       | 21.9               | 15.8       | 22.8       |
| 12   | 39.8                 | 18.2       | 16.4       | 48.3        | 38.3       | 34.9       | 21.9               | 14.6       | 18.1       |
| 13   | 33.7                 | 18.0       | 19.8       | 44.9        | 33.8       | 34.1       | 20.8               | 14.6       | 19.2       |
| 14   | 33.8                 | 18.5       | 23.9       | 38.2        | 33.7       | 37.1       | 21.8               | 12.1       | 15.9       |
| 15   | 41.6                 | 13.4       | 19.6       | 36.2        | 30.0       | 30.5       | 20.3               | 12.3       | 15.1       |
| 16   | 37.7                 | 13.5       | 8.8        | 39.9        | 16.9       | 26.7       | 21.1               | 23.9       | 16.2       |
| 17   | 25.2                 | 15.5       | 9.8        | 40.1        | 13.8       | 21.7       | 16.5               | 19.5       | 15.4       |
| 18   | 21.4                 | 18.4       | 9.0        | 42.2        | 12.2       | 18.1       | 27.2               | 16.9       | 17.3       |
| 19   | 18.9                 | 15.5       | 10.1       | 27.9        | 18.6       | 11.9       | 25.1               | 11.8       | 21.4       |
| 20   | 22.1                 | 9.4        | 13.6       | 27.7        | 17.4       | 3.2        | 24.4               | 12.2       | 18.2       |
| 21   | 20.4                 | 13.4       | 9.9        | 30.0        | 19.2       | 6.9        | 20.7               | 22.4       | 13.8       |
| 22   | 18.9                 | 14.1       | 5.5        | 32.8        | 23.8       | 10.2       | 18.5               | 9.2        | 12.9       |
| 23   | 27.2                 | 14.4       | 1.4        | 32.1        | 29.7       | 7.1        | 18.4               | 15.6       | 9.0        |
| 24   | 16.8                 | 14.3       | 2.9        | 31.6        | 19.9       | 3.3        | 17.3               | 14.6       | 12.2       |
| 25   | 16.3                 | 12.2       | 2.3        | 25.0        | 17.4       | 6.6        | 20.7               | 15.3       | 15.1       |
| 26   | 17.5                 | 10.4       | 3.6        | 15.7        | 3.6        | 5.3        | 21.2               | 13.5       | 14.6       |
| 27   | 17.8                 | 11.3       | 3.7        | 27.9        | 5.8        | 3.4        | 19.6               | 13.6       | 9.4        |
| 28   | 8.3                  | 7.3        | 2.6        | 27.1        | 4.1        | 2.0        | 26.8               | 10.3       | 13.7       |
| 29   | 13.6                 | 4.8        | 0.3        | 28.9        | 2.9        | 1.7        | 19.4               | 7.6        | 17.5       |
| 30   | 13.4                 | 0.7        | 0.2        | 21.1        | 1.0        | 0.2        | 21.7               | 14.8       | 16.6       |

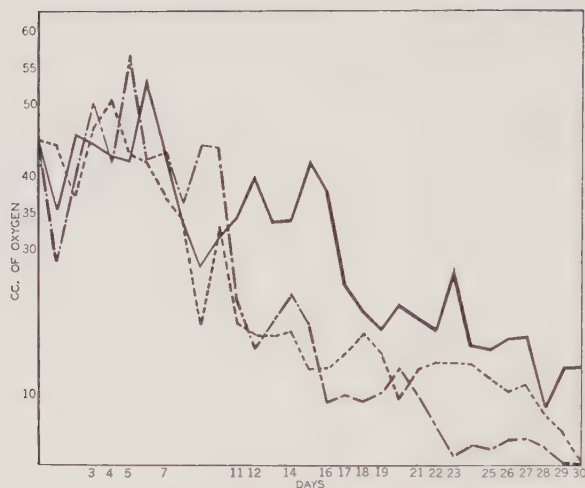


FIG. 5. The effect of a limited supply of oxygen on the catalase activity of Golden Bowl, 1934 crop. Solid line represents imbibed seeds immersed under 5 mm. of water, line of short dashes seeds immersed under 100 mm., and line of long and short dashes seeds under 155 mm.

was evidently correlated with the oxygen supply. The seeds submerged at a depth of 100 mm. and 155 mm. showed a much greater decrease of catalase activity over the later period of the experiment than did those at a depth of 5 mm. In all cases the decline in the catalase activity was well under way in the seeds subjected to 100 mm. and 155 mm. by the fifteenth day. The Golden Bowl seeds of the 1934 crop, at all three depths, showed at some stage in the course of the experiment a greater catalase activity than was present at the beginning. This was shown by the seeds of the 1936 crop of the same species at the 5-mm. depth and not at all by the seeds of variety Dark Red.

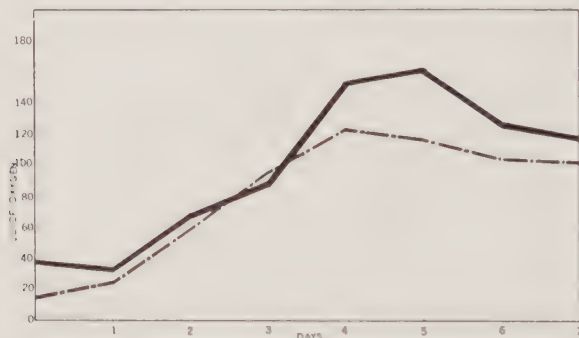


FIG. 6. The catalase activity of seeds of variety Dark Red treated with oxygen: solid line represents imbibed seeds, and the broken line imbibed and held under 155 mm. of water for 30 days before testing.



At the end of the thirty-day period the seeds of this variety showed a greater catalase activity than did those of Golden Bowl.

Seeds of variety Dark Red after being submerged thirty days under a 155-mm. layer of water were removed and placed under ideal germinating conditions with a steady stream of oxygen passing over them; the seeds thus treated gave a 50 per cent. germination. Catalase determinations of the germinating seeds were made on seven consecutive days. Figure 6 shows a comparison of the catalase activity of seeds submerged for 30 days, and of imbibed seeds when placed under oxygen. The submerged seeds show an increase in catalase activity and follow the same general pattern as do the imbibed seeds.

#### TEMPERATURE EFFECTS ON OXIDASE

Imbibed seeds were placed on moist filter paper and cellucotton in sterile Petri dishes and subjected to 8° and 47° C. for a period of 7 days. Oxidase determinations were made daily using 12 seeds of the Golden Bowl and 24 seeds of Dark Red. The results show (table V) that oxidase, like catalase, is destroyed or inactivated at 47° C. The amount of oxidase fluctuates at 8° C., showing no great increase, and reaches its lowest level on the fourth day in the seeds of Golden Bowl and on the seventh day in those of Dark Red.

TABLE V

TEMPERATURE EFFECTS ON OXIDASE ACTIVITY OF HIBISCUS SEEDS. MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY FOR TWO HOURS ON SEVEN CONSECUTIVE DAYS

| DAYS | GOLDEN BOWL, 12 SEEDS |            | DARK RED, 24 SEEDS |            |
|------|-----------------------|------------|--------------------|------------|
|      | 8° C.                 | 47° C.     | 8° C.              | 47° C.     |
|      | <i>cm.</i>            | <i>cm.</i> | <i>cm.</i>         | <i>cm.</i> |
| 0    | -0.51                 | -0.51      | -0.49              | -0.49      |
| 1    | -0.25                 | -0.00      | -0.27              | -0.00      |
| 2    | -0.41                 | -0.00      | -0.21              | -0.00      |
| 3    | -0.21                 | -0.00      | -0.33              | -0.00      |
| 4    | -0.14                 | -0.00      | -0.29              | -0.00      |
| 5    | -0.20                 | -0.00      | -0.13              | -0.00      |
| 6    | -0.19                 | -0.00      | -0.15              | -0.00      |
| 7    | -0.33                 | -0.00      | -0.07              | -0.00      |

#### EFFECT OF OXYGEN AND NITROGEN ON OXIDASE

The imbibed seeds were placed in bottles in the same manner as in the catalase experiment and subjected to identical conditions. Daily determinations were made using 12 of the larger variety and 24 seeds of the smaller variety as in the temperature tests.

The seeds subjected to the steady stream of oxygen showed rapid germination, but the oxidase content seemed to fluctuate during the early part of the experiment. The oxidase activity reached its maximum degree in

Golden Bowl seeds on the 6th day and in the seeds of Dark Red on the 5th day. The results indicate that the oxidase content does vary with germination of the seeds.

TABLE VI

OXYGEN EFFECTS ON OXIDASE ACTIVITY OF HIBISCUS SEEDS. MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY FOR TWO HOURS ON SEVEN CONSECUTIVE DAYS

| SEEDS                     | DAYS  |       |       |       |       |       |       |       |
|---------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
|                           | 0     | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
|                           | cm.   | cm.   | cm.   | cm.   | cm.   | cm.   | cm.   | cm.   |
| Golden Bowl .....<br>(12) | -0.51 | -0.22 | -0.37 | -0.41 | -0.30 | -0.33 | -0.72 | -0.37 |
| Dark Red .....<br>(24)    | -0.49 | -0.24 | -0.36 | -0.24 | -0.52 | -0.92 | -0.68 | -0.61 |

Only a few of the Golden Bowl seeds showed any visible signs of germination when subjected to nitrogen and the oxidase content, or activity, showed great fluctuation. The seeds of Golden Bowl showed a greater oxidase activity on the 5th day than did those treated with oxygen; this was not true for variety Dark Red although the high point occurred on the 5th day as it did in the oxygen-treated seeds.

TABLE VII

NITROGEN EFFECTS ON OXIDASE ACTIVITY OF HIBISCUS SEEDS. MANOMETER READING EXPRESSED IN CENTIMETERS OF MERCURY FOR TWO HOURS ON SEVEN CONSECUTIVE DAYS

| SEEDS                     | DAYS  |       |       |       |       |       |       |       |
|---------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
|                           | 0     | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
|                           | cm.   | cm.   | cm.   | cm.   | cm.   | cm.   | cm.   | cm.   |
| Golden Bowl .....<br>(12) | -0.51 | -0.23 | -0.67 | -0.15 | -0.75 | -0.94 | -0.41 | -0.41 |
| Dark Red .....<br>(24)    | -0.49 | -0.16 | -0.44 | -0.24 | -0.16 | -0.60 | -0.52 | -0.56 |

#### THE OXIDASE ACTIVITY OF SUBMERGED SEEDS

Imbibed seeds were placed in tubes containing sterile water as in the catalase experiment and under identical conditions. Twelve seeds were used for the daily oxidase determination of Golden Bowl and 24 seeds for the other species. In both varieties the first part of the period was marked by considerable fluctuation of oxidase activity in the seeds at all three depths. After the 15th day the oxidase activity decreased consistently.

TABLE VIII

OXIDASE ACTIVITY OF HIBISCUS SEEDS SUBMERGED IN WATER. MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY FOR TWO HOURS ON THIRTY CONSECUTIVE DAYS

| DAYS | GOLDEN BOWL, 12 SEEDS |            |            | DARK RED, 24 SEEDS |            |            |
|------|-----------------------|------------|------------|--------------------|------------|------------|
|      | 1934 CROP             |            |            | 1936 CROP          |            |            |
|      | WATER DEPTH           |            |            | WATER DEPTH        |            |            |
|      | 5 MM.                 | 100 MM.    | 155 MM.    | 5 MM.              | 100 MM.    | 155 MM.    |
|      | <i>cm.</i>            | <i>cm.</i> | <i>cm.</i> | <i>cm.</i>         | <i>cm.</i> | <i>cm.</i> |
| 0    | -0.51                 | -0.51      | -0.51      | -0.49              | -0.49      | -0.49      |
| 1    | -0.48                 | -0.67      | -0.71      | -0.75              | -0.46      | -0.63      |
| 2    | -0.41                 | -0.31      | -0.37      | -0.83              | -0.10      | -0.23      |
| 3    | -0.42                 | -0.64      | -0.41      | -0.41              | -0.06      | -0.39      |
| 4    | -0.30                 | -0.25      | -0.30      | -0.79              | -0.51      | -0.35      |
| 5    | -0.25                 | -0.57      | -0.25      | -0.41              | -0.25      | -0.35      |
| 6    | -0.34                 | -0.24      | -0.22      | -0.75              | -0.61      | -0.27      |
| 7    | -0.30                 | -0.40      | -0.28      | -1.13              | -0.25      | -0.35      |
| 8    | -0.64                 | -0.40      | -0.22      | -1.00              | -0.51      | -0.46      |
| 9    | -0.68                 | -0.16      | -0.18      | -0.67              | -0.67      | -0.27      |
| 10   | -0.71                 | -0.28      | -0.17      | -1.34              | -0.58      | -0.55      |
| 11   | -0.47                 | -0.36      | -0.14      | -0.54              | -0.32      | -0.59      |
| 12   | -0.41                 | -0.24      | -0.14      | -0.58              | -0.34      | -0.59      |
| 13   | -0.52                 | -0.12      | -0.07      | -0.40              | -0.35      | -0.43      |
| 14   | -0.23                 | -0.20      | -0.17      | -0.52              | -0.25      | -0.07      |
| 15   | -0.31                 | -0.36      | -0.14      | -0.66              | -0.19      | -0.23      |
| 16   | -0.20                 | -0.24      | -0.17      | -0.54              | -0.22      | -0.15      |
| 17   | -0.15                 | -0.24      | -0.17      | -0.33              | -0.18      | -0.15      |
| 18   | -0.11                 | -0.28      | -0.18      | -0.41              | -0.29      | -0.23      |
| 19   | -0.22                 | -0.24      | -0.18      | -0.66              | -0.26      | -0.17      |
| 20   | -0.19                 | -0.16      | -0.17      | -0.54              | -0.09      | -0.15      |
| 21   | -0.22                 | -0.24      | -0.17      | -0.54              | -0.15      | -0.15      |
| 22   | -0.27                 | -0.29      | -0.15      | -0.58              | -0.00      | -0.07      |
| 23   | -0.42                 | -0.12      | -0.14      | -0.37              | -0.00      | -0.15      |
| 24   | -0.22                 | -0.16      | -0.11      | -0.41              | -0.18      | -0.27      |
| 25   | -0.29                 | -0.16      | -0.14      | -0.16              | -0.10      | -0.11      |
| 26   | -0.11                 | -0.28      | -0.14      | -0.16              | -0.19      | -0.11      |
| 27   | -0.21                 | -0.08      | -0.14      | -0.46              | -0.28      | -0.19      |
| 28   | -0.19                 | -0.16      | -0.14      | -0.50              | -0.11      | -0.15      |
| 29   | -0.20                 | -0.28      | -0.14      | -0.62              | -0.12      | -0.15      |
| 30   | -0.36                 | -0.24      | -0.12      | -0.75              | -0.19      | -0.15      |

The seeds at the greater depth showed a greater decrease than did those at the 5 mm. depth. Some of the submerged seeds were able to push the radicle through the seed coat. Where the oxidase is present in small amounts, this lack of uniformity in the degree of germination in the individual seeds may be responsible for some of the fluctuation if the oxidase content is influenced by germination as the results indicate (table VI, fig. 7).

### Discussion

Catalase activity in the seeds of Hibiscus varieties Golden Bowl and Dark Red is depressed in the early stage of germination and then increases until



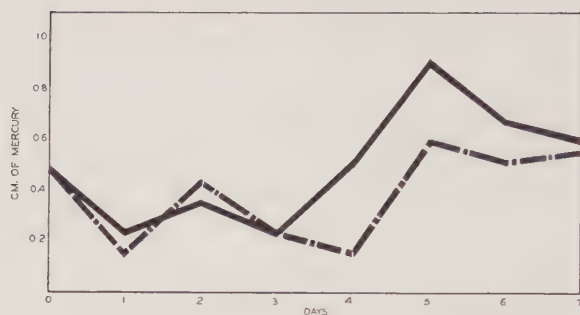


FIG. 7. The oxidase activity of seeds of variety Dark Red: solid line represents the imbibed seeds treated with oxygen, and the broken line seeds treated with nitrogen. The pressures are negative.

the food supply is apparently exhausted. This depression in the catalase activity is not in accordance with the findings of CROCKER and HARRINGTON (6) for grass seeds, but does agree with the work of RHINE (26) for wheat, feterita, clover, mustard, radish, and buckwheat, and of LANTZ (17) for corn. RHINE concluded that catalase increased with the need of the plant to destroy the injurious substances produced in respiration, and thus these by-products stimulated the production of catalase. She explained this decrease in the first stages of germination by assuming that the catalase reserve was used up, in attacking the respiratory products, faster than it

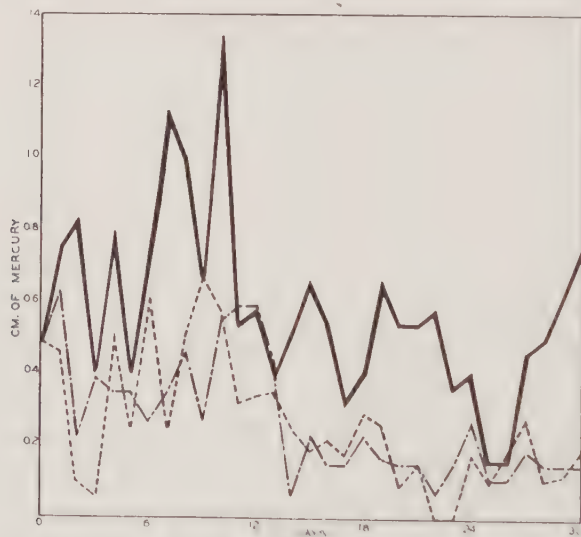


FIG. 8. The effect of a limited supply of oxygen on the oxidase content of imbibed seeds of variety Dark Red; solid line represents seeds immersed under 5 mm. of water, line of short dashes 100 mm., and line of long and short dashes 155 mm. The pressures are negative.

was produced. The increased stimulation of the accumulated respiratory by-products would, in a very short time, result in an increased production of catalase. The time required by the Hibiscus seeds to reach this low point of catalase activity depends on the speed of germination which in turn is dependent upon the external and internal conditions of the seeds.

The effect of the external conditions on the speed and the amount of catalase activity in the Hibiscus seeds is shown in the temperature studies in table I and figures 1 and 2. The seeds subjected to 8° C. required a longer period for the decrease in the catalase activity to occur than did those at 20° and 27° C. At the end of the seven-day period at this low temperature there was no evidence of the radicles pushing through the seed coats. The rate of increase of the catalase after the lowest point is reached is very slow in contrast to that in the actively germinating seeds. The lowest point of the catalase activity in the seeds placed at 20° and 27° C. is reached on the second or third day; there is then a sharp increase, reaching the peak during the 5th to 7th day. There is a slight difference in the catalase activity of the two species at 37° C. Variety Dark Red shows a decrease on the second day, with a very slight increase from the 2nd through the 5th, then a gradual decline. A few of these seeds were able to push the radicle through the seed coat at this temperature. The catalase activity of the seeds of Golden Bowl either reached the early decrease before the end of 24 hours or the seeds were unable to germinate at this temperature. At 47° C. in both varieties of seeds there is an abrupt drop in the catalase content beginning with the first day, while at 42° C. the trend is downward but at a slower rate.

The decrease and the lack of increase at the higher temperatures may be attributable to a combined set of factors. At 42° and 47° C. there is no evidence of germination, and since there seems to be some correlation between the germination and rate of catalase activity, this may be responsible for the absence of an increase in catalase activity. On the other hand, catalase shows its similarity to other enzymes in its reaction to several external conditions. There is no one temperature at which inactivation of all enzymes takes place, yet the temperature, time of exposure to heat, and the presence of other substances seem to play an important part in the rate of inactivation (12, 21). MORGULIS and BEBER (21) state that catalase is slowly inactivated at temperatures below 50° C.; inactivation becomes very rapid as the temperature is raised to 55° C. or above, especially at a pH of less than 6. Considering enzymes in general, FALK (12) finds that they show their greatest catalytic activity at temperatures near 40° C. At higher temperatures he thinks it is probable that inactivation of the enzymes takes place with sufficient rapidity to cause an apparent decrease in catalytic action. Investigators using the catalase extract have been able

to subject it to a higher temperature for a short period of time without completely destroying the catalytic activity. This shows the importance of the length of time the enzyme is exposed to a given temperature. An increase in the catalytic activity of a yeast suspension was obtained by EULER and BLIX (10) by heating for one hour at a temperature of 55°–60° C. They attributed this increased activity to the destruction of an inhibitory substance. CROCKER and HARRINGTON (6) heated air dry seeds of *Amaranthus retroflexus* for 4 hours at a temperature of 81° C. with a loss of only 40 per cent. of its original catalase activity, while the vitality of the seed was reduced 75 per cent. They found the reverse to be true for the seeds of Johnson grass. Thus the temperature which will inactivate catalase is determined by the source of the catalase and the conditions under which it is exposed.

The Hibiscus seed subjected to a steady flow of oxygen germinated rapidly at 25°–27° C. and the catalase activity, after decreasing for a short period, increased rapidly as is shown in table II and figure 3. Submerged seeds with a limited supply of oxygen showed similar reaction but there is a decrease in the amount of catalase activity (table IV, fig. 4). This decrease is greater in the seeds submerged at depths of 100 and 155 mm. than at 5 mm. When the oxygen is completely eliminated by passing a steady stream of nitrogen (table III, fig. 5) over the imbibed seeds there is a decrease in the catalase activity of the seeds of Dark Red; in Golden Bowl there is first a decrease, then a slight increase, and finally a second decrease which is typical of germinating seeds. A few of the Golden Bowl embryos were able to push the radicle through the seed coat; none of the Dark Red embryos was able to do so. MARKS and FOX (19) found that catalase extract obtained from muscle, stored under nitrogen, was inactivated at a slower rate than when kept under oxygen or air. It is probable that the effect on the increase of catalase activity is indirect, since germination proceeds rapidly when the seeds are exposed to air or excess oxygen and favorable temperature conditions, while the germination is depressed when the seeds are subjected to a very limited supply of oxygen or none at all. The amount of decrease found over a period of 30 days may be caused by lack of oxygen. When seeds of variety Dark Red were kept under 155 mm. of water for 30 days, then removed and subjected to a steady stream of oxygen and favorable germinating conditions, 50 per cent. of the seeds germinated and the catalase activity of the germinating seeds increased as shown in figure 6. The increase follows the same general course as for imbibed seeds. MORINAGA (22) found that in rice germinated aerobically there was an increase in catalase while in anaerobic germination there was a decrease in the amount of catalase and also in the length of the radicle.



Catalase activity in germinating seeds decreases when the food supply is exhausted according to the findings of LANTZ (17) and DELEANO (7). This is shown to be the case in the Hibiscus seeds. CROCKER and HARRINGTON (6) found that the catalase activity of the embryo of Stoner wheat and Sudan grass was 28 to 29 times that of the endosperm. They explain this by claiming that the endosperm is composed mainly of dead cells with the exception of the aleurone layer. HEINICKE (16) cites the work of AUCHTER and others in which a correlation was found between the nitrogen content of the tissues and its ability to destroy  $H_2O_2$ , while an increase in carbohydrate content without further addition of nitrogen depresses catalase. HEINICKE (15, 16) noted that the catalase activity in apple-leaf tissue was influenced by the factors which affected the nutritive or physiological conditions. He found that nitrogenous fertilizers increased the catalase activity of leaves from trees growing in sod. Bark of the roots had a higher activity than did bark from the trunk and the bud tissues were the most active of any on the tree. Strains of corn high in oil content displayed the greatest catalase activity, and the high protein strains ranked second when compared with low fat and low protein strains, according to LANTZ (17). BILETZKY (4) contends that more attention should be given to the factors concerned in the production of the plant and their relation to the catalase content. His findings correspond with others who state that nitrogen fertilizer tends to increase the catalase content. Since catalase apparently increases through the stages in germination when the stored food material is being converted into soluble form, with a subsequent decrease when this material is exhausted, the formation of the catalase may be brought about by the breaking up of the stored food. DOYLE (9) states that there is a temptation to look upon catalase as a labile metabolic by-product rather than as an enzyme with a fundamental physiological function.

The oxidases in general were found to react to the various environmental conditions in much the same way as did the catalase. The amount of oxidase present in the seeds before treatment was negligible, consequently it was necessary to increase the number of seeds used for the determinations. In a seed with such an impervious seed coat oxidation is doubtless reduced to a minimum; thus the imbibed seeds have a greater oxidase content than do the dry seeds. The oxidase increased in the seeds which were able to germinate. CROCKER and HARRINGTON (6) found a greater oxidase activity in coleoptiles and roots than in the whole seeds of Johnson grass, but believed the ratio was no greater than would be expected from the comparison of embryo ends with caryopses.

Oxidase was similar to catalase in its response to low and high temperatures. The oxidases were inactivated at 47° C. by the end of a 24-hour

period. At 8° C. there was no great increase in the amount of oxidase over a period of 7 days.

The seeds subjected to an abundant supply of oxygen showed considerable fluctuation in the oxidase activity during the first few days but reached a very high peak on the 5th day for variety Dark Red and on the 6th day for Golden Bowl. The irregularities found during the first part of the period would undoubtedly be smoothed out with a greater number of determinations. In general, the oxidase increased during the period of germination. When the oxygen supply is limited, or lacking completely, there is a remarkable amount of fluctuation in the oxidase content. The results (table VIII) indicate a greater difference in the oxidase content in seeds of variety Dark Red than in those of Golden Bowl when the supply of oxygen is limited. At the shallower submerged depth, seeds of variety Dark Red showed a much higher oxidase content than when kept at greater depths, and at all depths oxidase decreased considerably after the 11th or 12th day. When the supply of oxygen was replaced by nitrogen the Golden Bowl seeds showed a greater power of germination and greater oxidase activity than did the seeds of variety Dark Red.

### Summary

1. The effect of temperature, an excessive supply of oxygen, a limited supply of oxygen, and the absence of oxygen on the catalase and oxidase activity of imbibed seeds of two varieties of Hibiscus, or rose mallow, was studied.

2. In all seeds capable of germination the catalase activity follows a very definite course. In the earliest stages of germination the catalase activity is depressed but soon increases rapidly as germination progresses. The oxidase content shows some fluctuation at first, then increases according to the rate of germination.

3. Temperature plays an important rôle in the germination of the seeds and in the activity of the enzymes. At low temperatures (8° C.) germination is slowed up as is catalase and oxidase activity. Medium temperatures (20°–27° C.) favor germination and likewise favor increased activity of oxidase and catalase. Temperatures above 40° C. inhibit germination of the seeds; correspondingly the enzymes not only fail to increase but decrease very rapidly.

4. An excess or adequate supply of oxygen favors germination of the seeds and also favors an increase in the activity of oxidase and catalase. When the supply is limited or inadequate there is a tendency for germination to be retarded or inhibited and this is accompanied by corresponding loss of activity of the enzymes.

5. There is a close relationship between the environmental factors and the activity of the oxidizing enzymes in rose mallow seeds.

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